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# CHITOSAN IS AN EFFICIENT TOOL FOR PRECISION AGRICULTURE AND SUSTAINABILITY: A REVIEW

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Chitosan, a biopolymer composed of D-glucosamine and N-acetyl-D-glucosamine units linked by 1,4glycosidic bonds, has garnered significant attention due to its versatile applications in agriculture and various industries. Its solubility in acidic environments, primarily due to the protonation of the -NH, groups, sets it apart from chitin and enhances its utility. Chitosan, a biodegradable and non-toxic alternative, is increasingly favored in agriculture for its environmental benefits and ability to enhance plant immunity, improve soil health, and leave no harmful residues. Unlike chemical pesticides and fertilizers, which can lead to soil and water contamination, pose health risks, and promote pest resistance, chitosan supports sustainable farming by boosting crop yield and quality while promoting beneficial microbial activity in the soil. This makes chitosan a safer and more sustainable option for modern agriculture. Here, we review explores chitosan's **ABSTRACT** structural attributes, natural origins and its multifaceted roles in enhancing seed germination, promoting plant growth, and improving yield attributes. Furthermore, the paper delves into the polymer's capacity to mitigate abiotic stresses such as drought, heat, and salinity, emphasizing its potential as a biostimulant and antitranspirant. The review also highlights chitosan's efficacy in plant disease management, including its antimicrobial properties against fungal, bacterial, and viral pathogens and its application in post-harvest disease control to extend the shelf life of produce. This comprehensive analysis underscores chitosan's promise as a pivotal agent in sustainable agriculture and plant protection.

Key words : Chitosan, Seed germination, Plant growth and yield, Abiotic stress, Plant disease management.

# Introduction

In the face of escalating environmental challenges and the urgent need for sustainable agricultural practices, the exploration of novel solutions has become imperative. Traditional agrochemicals, while effective in boosting crop yields and controlling pests and diseases, often come with detrimental environmental and health consequences. This has prompted a paradigm shift towards eco-friendly alternatives that prioritize both agricultural productivity and environmental stewardship.

Chitosan, a derivative of chitin found abundantly in the exoskeletons of crustaceans and the cell walls of fungi, has garnered significant attention for its multifaceted applications in agriculture. It is one of the most preferred biopolymers due to its biocompatibility, antioxidant, anticancer, biodegradability, antimicrobial, and non-toxic properties, as well as being an economical material produced from waste resources such as seafood shells (Dash *et al.*, 2011; Shukla *et al.*, 2013; Vroman and Tighzert, 2009). Unlike synthetic agrochemicals, chitosan offers a sustainable and biodegradable option, aligning with the principles of green chemistry and the circular economy. Research on chitosan has increased significantly over the past decade due to its diverse applications in fields such as plant sciences (Kaya *et al.*, 2009; Kaya *et al.*, 2015 and Rinaudo, 2009) and medical sciences (Shamov *et al.*, 2002).

The journey of chitosan from waste material to agricultural marvel begins with its extraction and purification from shrimp and crab shells, a process that not only repurposes industrial by-products but also reduces waste and environmental pollution. This inherent sustainability underscores chitosan's appeal as a cornerstone of environmentally responsible agriculture. In plants, chitosan is largely used to mimic biotic and abiotic stresses. The first study of chitosan as an antipathogen in plants was reported by Allan and Hadwiger (1979), demonstrating its fungicidal effects on various fungal cell wall compositions. The improvement of plant defense systems through the application of chitin and chitosan in both monocotyledons and dicotyledons has become a central focus in multi-disciplinary research (Barber et al., 1989). Chitosan functions as a biofungicide, bio-bactericide and bio-virucide, which stimulates plant defense mechanisms against pathogens, thereby inducing the immune systems of plants, fruits, and vegetables (Kaya et al., 2018; Allan and Hadwiger, 1979; Chirkov et al., 1994 and Li et al., 2013).

Beyond its ecological merits, chitosan boasts an impressive array of properties that make it a versatile tool in agricultural management. Its ability to form biocompatible films makes it an ideal candidate for crop protection, while its cationic nature enables interactions with plant surfaces and microbial pathogens. Additionally, chitosan's immune stimulant properties can enhance plant defense mechanisms, bolstering resilience against biotic and abiotic stresses.

The applications of chitosan in agriculture are manifold and continue to expand with ongoing research and innovation. From its role as a bio-pesticide in pest and disease management to its use as a bio-stimulant for enhancing plant growth and productivity, chitosan offers a holistic approach to sustainable crop management. The growing demand for food has also stimulated increased use of industrial fertilizers, which cause serious environmental imbalances and have catastrophic effects on human health. Therefore, the use of chitosan as a biofertilizer is being considered. Chitosan has been reported to positively affect rhizobacteria growth, forming a symbiotic relationship with growth-promoting rhizobacteria, thereby enhancing germination rates and improving plant nutrient uptake (Agbodjato *et al.*, 2016).

However, despite its immense potential, the widespread adoption of chitosan in agriculture faces

several challenges. These include issues related to formulation stability, efficacy under variable environmental conditions, and regulatory hurdles. Addressing these challenges requires concerted efforts from researchers, policymakers and industry stakeholders to facilitate the integration of chitosan-based solutions into mainstream agricultural practices.

In light of these considerations, this review aims to provide a comprehensive synthesis of the current state of knowledge regarding the applications, mechanisms of action, formulation strategies, and challenges associated with chitosan in agriculture. By critically evaluating existing literature and identifying areas for future research and development, this review seeks to contribute to the ongoing dialogue on sustainable agricultural innovation and the role of chitosan therein.

### Structure and Natural Origins of chitosan

Chitosan is a linear polymer composed of two subunits, D-glucosamine and N-acetyl-D-glucosamine, joined by 1,4-glycosidic bonds. The overall structure of chitosan comprises three rings. It possesses three functional groups: amino groups, primary and secondary hydroxyl groups, facilitating its chemical modification. These functional groups significantly influence chitosan's solubility and mechanical properties. Moreover, chitosan contains -1,4 glycosidic linkages. In acidic aqueous environments, chitosan exhibits higher solubility than chitin. The primary reason for chitosan's solubility lies in the protonation of -NH, at the C-1 position of the Dglucosamine repeat unit, transforming the polysaccharide into a polyelectrolyte under acidic conditions. Given its solubility properties, chitosan finds applications in various sectors such as agriculture, medicine, process engineering, and industries. Chitosan can be directly extracted from various fungi, or it can be produced by extracting chitin and then subjecting it to a deacetylation process. In the fungal kingdom, chitin is more commonly produced than chitosan, being synthesized by zygomycetes, ascomycetes, basidiomycetes, deuteromycetes and phycomycetes. In contrast, chitosan is exclusively found in the cell walls of certain fungal taxa, particularly zygomycetes. Extracting chitosan from fungal biomass offers significant advantages as it can be carried out at any time and is not subject to seasonal changes. A process akin to the extraction of chitosan from crustaceans is employed to extract chitin from fungi, where their cell walls serve as the sole source. Therefore, using only fungi that currently produce the desired product is more cost effective (Sharif et al., 2018). Another very important and abundant source of chitin comes from the



Fig. 1 : Design of chitin and chitosan structure, chemistry and functional groups (Aranaz *et al.*, 2021).

exoskeletons of crustaceans; mainly from a variety of marine crustaceans such as shrimp, crabs and lobsters. Currently, the main source of chitin comes from waste from the shrimp industry, where exoskeletons are obtained to obtain chitin and calcium. Chitosan is obtained through a chemical process of N-deacetylation, where the cationic nature of chitosan is owed to the free amino group left by the partial removal of the acetyl group of chitin (Dima *et al.*, 2017).

### Mechanism of Chitosan action in Plant responses

To date, the mode of action of chitosan in plants remains unclear. However, several reports suggest that chitosan elicits several defense responses in plants (Fan *et al.*, 2021; Zhang *et al.*, 2022).

#### **Signal Perception and Transduction**

## Signal Perception by Chitosan Induction

A chitosan-binding glycoprotein in the lectin family has been identified from mustard leaves (Brassica campestris) (Yang et al., 2017). Consequently, the isolation of vesicles from Mimosa pudica and Cassia fasciculata indicated rapid activation of the H+-ATPase plasma membrane, thereby revealing chitosan receptor molecules (Smith and Brown, 2018). Additionally, a knockout mutant in Arabidopsis thaliana showed that chitosan could induce a receptor-like kinase gene, the mitogen-activated protein kinase pathway, and lysin motif receptor-like kinase as a chitin elicitor receptor kinase 1 (CERK1), which can bind with both chitin and chitosan (Lee et al., 2019). However, a report involving A. thaliana seedlings stated that the chitosan receptor did not involve CERK1 and also reacted through a CERK1-independent pathway (Kim et al., 2020). Therefore, signal perception induced by chitosan remains to be clarified.

# **Signal Transduction**

Chitosan application could stimulate defense



**Fig. 2 :** Signal transduction by chitosan in plant cell subjected to stresses (Pongprayoon *et al.*, 2020). JA (jasmonic acid), SA (salicylic acid), ABA (abscisic acid).

responses via hydrogen peroxide  $(H_2O_2)$  through the octadecanoid pathway and nitric oxide (NO) in the chloroplast, MAP-kinase activation, oxidative production, and hypersensitive responses (Perez et al., 2021). In chitosan-treated plants, these signal molecules affect adaptive mechanisms in response to biotic and abiotic stresses. The interaction between chitosan and plant cells is initiated when chitosan binds to specific receptors, eliciting secondary messengers such as H<sub>2</sub>O<sub>2</sub>, calcium ions  $(Ca_{2}^{+})$ , NO and phytohormones inside the cell to induce physiological responses (Gonzalez et al., 2018; Perez et al., 2021). Hydrogen peroxide acts as a signal molecule to induce resistance to osmotic stress in the 'Leung Pratew123' ('LPT123') rice (Oryza sativa) and the mutated line LPT123-TC171, by enhancing plant growth and maintaining photosynthetic pigments under osmotic stress (Chowdhury et al., 2017).

Chitosan induces  $Ca_2^+$  in plant species by regulating callose synthase activity (Lopez *et al.*, 2018; Wang and Xu, 2019), resulting in  $Ca_2^+$ -mediated programmed cell death in soybean (*Glycine max*) cells (Kumar *et al.*, 2020). Additionally, NO-signaling has been found in pearl millet (*Pennisetum glaucum*) seedlings treated with chitosan (Garcia *et al.*, 2020).

Chitosan also elicits the accumulation of jasmonic acid (JA) in several plants such as tomato (*Solanum lycopersicum*) (Johnson *et al.*, 2018), French bean (*Phaseolus vulgaris*) (Rodriguez *et al.*, 2019) and rapeseed (*Brassica napus*) (Nguyen and Tran, 2020). In rice (*O. sativa*) seedlings, chitosan induced an increase in JA and the accumulation of 12-oxophytodienoic acid via the octadecanoid pathway (Martinez *et al.*, 2019). Additionally, abscisic acid (ABA) also increased via  $H_2O_2$  signaling, leading to stomatal closure and reduced water usage in plants under abiotic stress (Perez *et al.*, 2021). Chitosan application induced ABA accumulation in leaf tissues and elicited resistance to tobacco necrosis virus (TNV) (Ochoa *et al.*, 2017). Moreover, JA and salicylic acid (SA) are plant hormones required for signal transduction leading to plant resistance to pathogens and insects (Chen *et al.*, 2020). JA mediates induced systemic resistance, while SA mediates systemic acquired resistance (SAR) (Smith and Johnson, 2021).

# Effect chitosan on seed germination

Chitosan has been explored for its potential role in enhancing seed germination. One mechanism by which chitosan may influence germination is by improving water retention in the seed microenvironment, ensuring consistent hydration levels critical for germination. Additionally, chitosan coatings on seeds have been found to enhance resistance to various stress factors, such as drought and salinity, which can inhibit germination. Moreover, chitosan's antimicrobial properties can protect seeds from fungal and bacterial pathogens that may impede germination or cause seed rot. By creating a protective barrier, chitosan helps safeguard seeds during the critical germination phase.

Pre-treatment with 0.5% chitosan increased the germination percentage, rate, and seedling vigor index, while also reducing the mean germination time and malondialdehyde levels in sweet corn (Behboud et al., 2020). Seeds pretreated with chitosan exhibited significantly higher expression levels of genes encoding dehydration-responsive transcription factors (DREB2, DREB4 and DREB5) and dehydrins (Y2K, Y2SK and SK2) compared to seeds without chitosan priming in white clover (Ling et al., 2022). High concentrations of chitosan hydrolysate completely inhibited seed germination. However, short-term treatment with high concentrations of chitosan hydrolysate stimulated seedling development, resulting in substantially longer roots in tomato (Timofeeva et al., 2022). Seed treatment with a magnetite suspension and chitosan solutions before planting resulted in increased plant growth, root, and shoot length of wheat. This increase was significantly dependent on the concentration of chitosan, with the chitosan and magnetite combination being 1.3 times more effective for seed germination than pure chitosan or magnetite alone (Parfenova et al., 2021).

Seed soaking with 0.15–0.20% chitosan had a beneficial effect on the seed germination of *Platycodon grandiflorus*, significantly promoting its germination rate, energy, index, as well as cotyledon and radicle length (Liu *et al.*, 2022). Seeds soaked with chitosan increased

the germination percentage of maize seedlings (Shao *et al.*, 2005). The application of chitosan increased the germination rate of cucumber, chilli, pumpkin and cabbage (Chandrkrachang, 2002). Seed priming with chitosan enhances seed germination and seedling vigor in pearl millet (Manjunatha *et al.*, 2008). Seeds soaked with chitosan increased the germination rate, length and weight of hypocotyls and radicles in rapeseed (Sui *et al.*, 2002). Treatment with 493 kDa chitosan improved growth in soybean sprouts (No *et al.*, 2003). Chitosan treatment (476 kDa) increased the total weight as well as the length and thickness of sunflower hypocotyls compared with the control (Cho *et al.*, 2008).

# Effect of chitosan on Plant growth, Yield attributes, and Physiological activities

Chitosan application has been shown to significantly enhance plant growth, yield attributes and physiological activities. It promotes seed germination, seedling vigor, root and shoot development, leading to improved water and nutrient uptake. This results in increased biomass, higher grain and fruit yield, and better fruit quality. Additionally, chitosan enhances chlorophyll content and leaf area, boosting photosynthesis and overall plant health. It also helps plants mitigate abiotic stresses such as drought, salinity, and temperature extremes, thereby improving resilience and productivity in various environmental conditions.

# Effects of Chitosan on Abiotic stresses

## Effect on Drought stress

Drought stress is one of the most significant environmental stressors, adversely affecting plant physiology, biochemical properties, and molecular traits (Lisar and Agdam, 2016). However, the application of chitosan prior to drought stress can stimulate plant growth and increase water and essential nutrient uptake by enhancing antioxidants to scavenge reactive oxygen species (ROS) (Guan et al., 2009). For instance, in apples, young seedlings subjected to continuous drought stress for 35 days exhibited enhanced antioxidant activity, reduced electrolyte leakage, and restored moisture content when foliar-sprayed with chitosan (Yang et al., 2009). Chitosan has also demonstrated the ability to induce resistance against drought stress in potato, moth orchid, rice, white clover and grapevine by stimulating antioxidant activities, increasing endogenous H<sub>2</sub>O<sub>2</sub> content, boosting endogenous chitosan activities, and fostering root system development (Jiao et al., 2012; Gu, 2011; Pongprayoon et al., 2013; Li et al., 2017). In the grapevine (V. vinifera) stem, chitosan concentration of 1.0% (w/v) induced drought tolerance by maintaining

Plant Species	Chitosan effect	Mode of Application	References
Rice (Oryza sativa L.)	Increased plant growth, higher photosynthesis rate	In vivo	Phothi and Theerakarunwon (2017)
Soybean (Glycine max)	Increased plant growth	Soil application	Chibu <i>et al.</i> (2002)
Rape (Brassica rapa L.)	Increased plant growth and content of leaf chlorophyll	Hydroponic pot application         Zong et al. (20)	
Maize (Zea mays L.)	Increased plant growth and grain weight	Biofertilization	Choudhary et al. (2017)
	Improved seed germination	In vivo	Shao <i>et al.</i> (2005)
	Improved seed germination and vigor index	In vivo	Saharan <i>et al</i> . (2016)
Soybean sprouts ( <i>Glycine max</i> )	Increased plant growth	In vivo	Lee et al. (2005)
Chickpea (Cicer arietinum)	Increased plant growth	In vivo	Mahdavi and Safari (2015)
	Increased seed germination and vigor index	In vivo	Anusuya and Nibiya (2016)

**Table 1 :** Effects of Chitosan on Growth, Yield and Physiological activities of cereals and legume crop.

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<b>Table 2.</b> Effects of Childsan on Growth Steld and Physiological activities of year	eranies
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Plant species	Chitosan effect	Mode of Application	References
Potato(Solanum tuberosum L.)	Increased tuber size	In vivo	Rodríguez et al. (2017)
	Increased plant growth and yield	In vitro and in vivo	Kowalski <i>et al.</i> (2006)
Tomato (Solanum lycopersicum)	Improved fruit quality and productivity	In vivo	Sathiyabama <i>et al.</i> (2015)
	Increased seed germination and vigor index	In vivo	Saharan et al. (2015)
Cabbage(Brassica oleracea)	Increased plant growth	In vivo	Spiegel <i>et al.</i> (1988)
Okra (Abelmoschus esculentus L.)	Increased plant growth, and yield	In vivo	Mondal <i>et al.</i> (2012)
Eggplant (Solanum melongena)	Increased plant growth, and yield	In vivo	Sultana <i>et al.</i> (2017)
Bean (Phaseolus vulgaris)	Increased leaf area, and carotenoids and chlorophylls levels	In vitro	Pereira <i>et al.</i> (2017)
Chilli (Capsicum frutescence L.)	Increased plant growth, yield, and thous and seed weight	In vivo	Akter <i>et al</i> . (2018)
	Increased leaf area, canopy diameter and plant height	In vivo	Chookhongkha <i>et al.</i> (2012)
Cucumber (Cucumis sativus)	Increased plant growth and improved quality	In vivo	Shehata <i>et al</i> . (2012)
Turmeric(Curcuma longa)	Increased plant growth and yield	In vivo	Anusuya and Sathiyabama (2016)

chlorophyll content under drought stress (Gornik and Romanowska, 2008). In cowpea (*Vigna unguiculata*), foliar application of chitosan improved growth and yield parameters in both drought stress and non-stress conditions (Farouk and Amany, 2012). Pre-treatment with chitosancontaining Hoagland's solution before drought stress increased the production of stress-responsive metabolites in white clover (*Trifolium repens*) (Li *et al.*, 2017). The application of chitosan three times by spraying before the flowering stage increased flowering by 50% and reduced the negative effects of drought stress in *Thymus daenensis* Celak (Bistgani *et al.*, 2017). Similarly, spraying chitosan three times before flowering enhanced plant growth in sweet basil (*Ocimum ciliatum and O*.

Table 3 : Ef	fects of Chitosan	on Growth,	Yield and F	Physiologica	al activities	of fruit crop.
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Plant species	Chitosan effect	Mode of Application	References
Strawberry (Fragaria annanasa)	Increased fruit yield and total antioxidant activities	In vivo	Rahman <i>et al.</i> (2018)
	Increased fruit yield	In vivo	Mutka et al., 2017
Strawberry (Fragaria annanasa)	Increased fruit yield and total antioxidant activities	In vivo	Rahman <i>et al.</i> (2018)
	Increased fruit yield	In vivo	Mutka <i>et al.</i> (2017)
Watermelon (Citrullus lanatus)	Increased plant growth	In vivo	González et al. (2017)
Mango (Mangifera indica)	Increased plant growth, fruit size and weight	In vivo	Zagzog <i>et al.</i> (2017)
Grapevine (Vitis vinifera L.)	Increased plant growth	In vivo	Barka <i>et al.</i> (2004)

Table 4 : Effects of Chitosan on Growth, Yield, and Physiological Activities of plant species

Plant species	Chitosan effect	Mode of	References
		Application	
Bell pepper(Capsicum annuum)	Increased fruit weight, diameter and yield	In vivo	Mahmood <i>et al.</i> (2012)
Ajowan ( <i>Carum copticum</i> )	Increased seed germination, vigor index, dry weight, and radical length	In vivo	Batool and Asghar (2013)
Artichoke (Cynara scolymus)	Improved seed germination and plant growth	In vivo	Ziani <i>et al.</i> (2010)
Coffee (Coffea arabica)	Increased plant height and leaf area	In vivo	Van <i>et al.</i> (2013)
Basil ( <i>Ocimum ciliatum</i> and <i>Ocimum basilicum</i> )	Increased plant growth and phenol content	In vivo	Pirbalout <i>et al</i> . (2017)
Phyla dulcis	Increased plant growth	In vitro	Sauerwein et al. (1991)
Freesia (Freesia corymbosa)	Increased plant growth	In vivo	Salachna and Zawadzinska (2014)
Gerbera jamesonii	Increased plant growth	In vivo	Wanichpongpan <i>et al.</i> (2000)
Dendrobium aggregatum	Increased plant growth	In vitro	Chandrkrachang (2002)
Cymbidium insigne	Increased plant growth	In vitro	Nahar <i>et al.</i> (2012)
Kemiri sunan (Reutealis trisperma)	Increased plant growth	In vivo	Irawati et al. (2019)
Scots pine (Pinus sylvestris L.)	Increased plant growth	In vivo	Trzcinska et al. (2017)

*basilicum*) (Pirbalouti *et al.*, 2017). Additionally, chitosan has been observed to promote ABA activity, a key regulator of stomatal aperture and transpiration rates during plant stress phases (Iriti and Faoro, 2008; Lim *et al.*, 2015). Consequently, chitosan could serve as a potential antitranspirant, aiding horticultural crops in managing drought stress.

# Effect on Heat stress

Heat stress presents a complex challenge in agricultural species, often occurring concurrently with drought stress, which complicates distinguishing between

the two stressors (McKersie and Lesheim, 2013). Studies suggest that in dry bean production under heat stress, treatment with chitosan could offer an effective approach to mitigate heat stress in late-sown plants (Ibrahim and Ramadan, 2015). A combination of zinc and humic acid with chitosan sprayed on dry bean leaves could confer resistance to heat stress (Shibashi *et al.*, 2011). Limited research is available on the involvement of chitosan in response to heat stress. However, the role of ABA (abscisic acid) in addressing heat stress is welldocumented (Ng *et al.*, 2014), particularly its function in inducing heat stress-related genes (Zhang *et al.*, 2005). Overexpression of ABF3 (abscisic acid-responsive element-binding factor 3) has been shown to confer tolerance to heat stress (Choi *et al.*, 2013). Correlating with previous reports, chitosan can induce stomatal closure by inducing ABA synthesis (Bittelli *et al.*, 2001). Hence, the use of chitosan may prove effective in alleviating high-temperature stress by stimulating ABA activity, which in turn induces the expression of defenserelated ABA-responsive genes in horticultural species.

#### Effect on Salt stress

Salinity affects the physiology and biochemistry of plant cells, potentially inhibiting water and nutrient uptake. Salt stress induces reactive oxygen species (ROS), leading to oxidative stress and lipid peroxidation, as indicated by malondialdehyde (MDA) accumulation, which further contributes to ion toxicity. Treatment with chitosan at low concentrations appears to mitigate the adverse effects of salt stress. Plants treated with chitosan demonstrate reduced oxidative stress through enhanced enzyme activities in safflower (Carthamus tinctorius L.) and sunflower (Helianthus annuus L.) (Jabeen and Ahmad, 2013). Chitosan pretreatment during salt stress increases the activities of antioxidant enzymes, resulting in lower MDA content in Carum copticum (Mahdavi and Rahimi, 2013), Plantago ovate (Mahdavi, 2013), Oryza sativa (Martínez et al., 2015), Vigna radiate (Ray, 2016) and Zea mays (Tawaha et al., 2018). Application of oligomeric chitosan to seeds also leads to a significant increase in antioxidant enzymes under salt stress in Triticum aestivum (Ma et al., 2012).

# Chitosan in plant disease management

# Chitosan for management of fungal diseases

Chitosan demonstrates efficacy in inhibiting various fungal activities, including hyphal growth, mycelial elongation, spore formation, germination, viability, germinal tube formation, and production of fungal virulence factors (Chakraborty et al., 2020). Its ability to penetrate phytopathogenic fungal plasma membranes depends on the membrane fluidity, with chitosan-sensitive fungi typically possessing polyunsaturated fatty acid-rich membranes, such as linoleic acid, resulting in high fluidity, while chitosan-resistant fungi often have membranes rich in saturated fatty acids like palmitic or stearic acid, leading to low fluidity (Guerrero et al., 2010). Chitosan induces morphological abnormalities such as excessive mycelial branching, abnormal shapes, swelling, and reduction in hyphal size in various fungi including F. oxysporum f. sp. cubense (Al-Hetar et al., 2011), F. solani f. sp. glycines (Prapagdee et al., 2007), Botrytis cinerea and Alternaria alternata (Oliveira et al., 2012). Additionally, chitosan causes cytological alterations, protoplasm dissolution and formation of large vesicles in fungi (Al-Hetar et al., 2011). Morphological changes such as the presence of large vesicles or empty cells devoid of cytoplasm have been observed in the mycelium of B. cinerea and F. oxysporum f. sp. albedinis (El Hassni et al., 2204 and Barka et al., 2004). Exposure to chitosan reduces spore production in treated fungi, and in some cases, complete suppression of sporulation is observed post-treatment. Furthermore, the size and shape of conidia



Fig. 3: An overview of chitosan-mediated plant growth regulation under stress conditions (Chakraborty et al., 2020).

of various fungi, such as *Ranunculus stolonifer*, *Penicillium digitatum* and *F. oxysporum* are significantly altered by chitosan (Baños *et al.*, 2004). Application of chitosan against *Fusarium oxysporum* f. sp. *cucumerinum* significantly decreases wilt disease severity compared to untreated *Fusarium* (Elagamey, 2022).

### Chitosan for management of bacterial diseases

Gram-positive and gram-negative bacteria have significantly different cell wall structures and surface polarity, which results in different sensitivity to chitosan (Ke et al., 2021). The cell wall of gram-negative bacteria is distinguished by the presence of lipopolysaccharides, which contain phosphate and pyrophosphate groups (Pasquina-Lemonche et al., 2020). This provides it with a high negative charge, making it more bound to chitosan (Cheung et al., 2015). While the cell wall of gram-positive bacteria contains polysaccharides associated with lipoteichoic and teichoic acids. Teichoic acid is negatively charged due to the presence of phosphate groups in its structure which gives it a small negative charge that makes it less bound to chitosan. The stopping of the teichoic acid biosynthesis pathway in Staphylococcus aureus, led to an increase in chitosan resistance (Raafat et al., 2005). Gram-negative bacteria are more hydrophilic than gram-positive bacteria and also have a thinner cell membrane (Chung et al., 2004). Chitosan has potent antibacterial activities against a variety of plant pathogenic bacteria like S. aureus (Tan et al., 2013), Streptomyces scabies (Beausejour et al., 2003), Ralstonia solanacearum (Farag et al. 2017), Xanthomonas spp. (Li et al. 2013), Pseudomonas spp. (Li et al., 2010) and Acidovorax spp. (Yang et al., 2014). The inhibitory activity of chitosan against bacteria varied with molecular weight (Li et al., 2016), concentration (Yang et al., 2014), solvent type (Rabea and Steurbaut, 2010), bacterial type (gram-positive/gram-negative) (Annaian et al., 2016), cell wall structure (Sapers, 1992), period of incubation and abiotic factors (Chung et al., 2013). Chitosan binds to the negatively charged surface of bacteria at low concentrations (less than 0.2 mg/ml) to cause agglutination, but at higher concentrations, the presence of more chitosan positive charges may have given the bacteria a net positive charge that keeps them suspended (Rabea et al., 2003). Moreover, Goy et al. (2016) suggest that chitosan is responsible for the hydrolysis of peptidoglycans, the main component of the bacterial cell wall, increasing electrolyte leakage and potentially causing the death of the plant pathogens. Additionally, Liang et al. (2014) reported that chitosan is the responsible substance for the destruction of the bacterial cell membrane, which causes death due to the leakage of intracellular substances. Chitosan applied to tomato plants inhibited the growth of Xanthomonas vesicatoria (Ramkissoon et al., 2016). Chitosan-protected cucumber from Pseudomonas syringae pv. lachrymans that causes bacterial angular leaf spot damage (Acar et al., 2008). Chitosan has decreased the disease incidence of broccoli that was infected with Pseudomonas fluorescens (Li et al., 2010). The disease index of watermelon seedlings infected with Acidovorax citrulli was significantly reduced by chitosan at 0.4 mg/ml (Li et al., 2013). Chitosan solution at 0.10 mg/ml markedly decreased the surviving cell number of Xanthomonas pathogenic bacteria isolated from different geographical origins compared with the control after 6 h of incubation, regardless of the bacterial strain (Li et al., 2008).

Overall, chitosan is a versatile and effective antibacterial agent against a variety of plant pathogenic bacteria, contributing to improved plant health and reduced disease incidence.

#### Chitosan for management of viral diseases

One of the most destructive plant diseases is the viral disease which causes serious damage to many plant species, affecting agroecosystems and food security. For that reason, searching for new eco-friendly application technologies to suppress the invasion of viral plant diseases is urgently needed to fulfill the nutrients required to feed the world's population (Dong and Ronald, 2019). Chitosan and its derivatives have been used as a promising and powerful tool against plant viruses. Chitosan has demonstrated antiviral activity against Potato virus X (PVX) through the possible mechanism of induced resistance and responsive defense mechanisms or the inhibition of systemic propagation of plant viruses in potato plants (Chirkov, 2002). Complete inhibition or suppression of systemic virus multiplication in the host plant has not clearly proven the capability of chitosan to stop the virus activation. However, the multiplication block may be due to the binding of chitosan molecules with the nucleic acid of a targeted virus, causing serious damage to the viral genome (Mansouri et al., 2004). Studies by Jia et al. (2016) explained the role of chitosan in inducing systemic acquired resistance in Arabidopsis plants infected with Tobacco mosaic virus (TMV) and which signaling pathways are involved in the processes of defense mechanisms. Their obtained results revealed that chitosan application induced TMV resistance through specific pathways in the plant. The induction in the Arabidopsis plants happened through the jasmonic acid pathwaydeficient (Arabidopsis plants jar1) and at the same time

Name of disease	Pathogen	Mode of action	References
Tomato wilt	Fusarium oxysporum f.sp. radices lycopersici	Chitosan was bound to negatively charged phospholipids that alter plasma membrane fluidity and induced membrane permeabilization.	Palma-Guerrero et al. (2010)
Potato dry rot	Fusarium sulphureum	Chitosan caused morphological changes such as intertwisting hyphal, distortion, and swelling with excessive branching, abnormal distribution of cytoplasm.	Li et al. (2009)
Early blight of potato, tomato, capsicum	Alternaria solani	Chitosan reduced hyphal growth, inhibited sporulation and spore germination, and induced morphological changes.	Guo <i>et al.</i> (2006) and Ramkissoon <i>et al.</i> (2016)
Cotton wilt	<i>Fusarium oxysporium</i> f. sp. vasinfectum		
Banana wilt	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i>	Chitosan formed abnormal shapes, vesicles and empty cells devoid of cytoplasm in the mycelia and agglomeration of hyphae.	Al-Hetar <i>et al.</i> (2011)
Soybean sudden death syndrome	Fusarium solani f. sp. glycines	Chitosan was able to induce the level of chitinase activity in soybean.	Prapagdee et al. (2007)
Cucumber wilt	<i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i>	Chitosan caused hindering of the Fusarium cell wall, disrupting DNA, disrupting structural and functional protein biosynthesis and influencing metabolic pathways.	Elagamey <i>et al</i> . (2022)
Root rot disease	Fusarium oxysporum	Chitosan covered the cell wall, causing membrane disruption and cell leakage.	Rabea et al. (2010)
Damping off diseases	Pythiumdebaryanum		
Anthracnose and ripen fruit rot in chilli	Colletotrichum capsici	Chitosan completely inhibited the mycelial growth of <i>C. capsici</i> by forming physical barriers around the penetration sites of the pathogen, preventing them from spreading to healthy tissues.	Akter <i>et al.</i> (2018)
Anthracnose of camellia	Colletotrichum camelliae	Chitosan enhanced the activity of $H_2O_2$ , the defense-related enzymes, such as polyphenol oxidase, peroxidase, catalase, phenylalanine ammonialyase.	Li and Zhu (2013)

Table 5 : Effects of Chitosan on Fungal diseases.

did not induce the salicylic acid pathway-deficient (Arabidopsis plants NahG). The application of chitosan as a protective and curative treatment against Alfalfa mosaic virus (AMV) on *Nicotiana glutinosa* plants under greenhouse conditions was studied by (Abdelkhalek *et al.*, 2021). They proved that the AMV concentration was significantly reduced through both protective and curative treatment with 70.43% and 61.65%, respectively. On the other hand, possible ways of inducing systemic resistance and responsive defense mechanisms were measured, resulting in an increase of total phenols and carbohydrates as well as phenylalanine amonialyase (PAL) and peroxidase.

Overall, chitosan's ability to induce systemic

resistance and interfere with viral propagation highlights its potential as a sustainable and effective measure against viral plant diseases, thereby contributing to improved plant health and agricultural productivity.

#### Chitosan for Post-Harvest disease control

Chitosan coatings applied to harvested fruits and vegetables can inhibit the growth of post-harvest pathogens, such as molds and decay causing fungi. These coatings create a protective barrier on the produce surface, reducing water loss, delaying senescence and extending shelf life.

Post-harvest diseases reduce the storage period and marketing life of citrus fruits. Anthracnose, caused by *Colletotrichum gloeosporioides*, is a unique post-harvest



Fig. 4: Antimicrobial mechanism of chitosan-based coating with antimicrobial agents (Kong *et al.*, 2010; Hosseinnejad and Jafari, 2016).

disease of citrus fruits. It is primarily prevented by applying synthetic fungicides (Zhang et al., 2011). However, in the last few decades, research has shown that treatment of navel oranges with 2% chitosan significantly reduces disease incidence and lesion diameter (Zeng et al., 2010). In pear fruit, chitosan and oligochitosan treatments greatly reduce disease incidence and inhibit lesion expansion caused by two fungal pathogens, Alternaria kikuchiana Tanaka and Physalospora piricola, with oligochitosan proving to be more effective in hindering the growth of P. piricola (Kennedy et al., 2010). In commercial wine grapes, chitosan effectively inhibits the growth of Botrytis cinerea in liquid culture and suppresses gray mold on detached grapevine leaves and bunch rot (Taylor et al., 2010). In mango, treatment with 1.0% and 0.75% chitosan significantly reduced incidence of post-harvest anthracnose disease caused by Colletotrichum gloeosporioides compared to control (Barman et al., 2017). Chitosan was effective in controlling blue mold decay of apple fruit caused by P. expansum. Both the lesion size of blue mold decay and patulin accumulation were significantly inhibited by chitosan treatment (Li et al., 2015). Chitosan caused a reduction in the germination of P. expansum by up to 78% and the length of the germ tube by 86%, compared to the control (Darolt et al., 2016).

Crab shell chitosan was effective in controlling postharvest fungal pathogens and maintaining pomegranate fruit quality (Munhuweyi *et al.*, 2017). Fruit coating is becoming one of the most popular methods to extend the commercial shelf life of fruits by delaying ripening, water loss, and decay (Baldwin *et al.*, 1997). Infections of *Cladosporium* spp. and *Rhizopus* spp. were reported to decrease in artificially inoculated strawberry fruit coated with chitosan and stored at 4–6°C for 20 days (Park et al., 2005). Recent studies have indicated that pre-harvest sprays of chitosan on strawberry plants reduced gray mold during fruit storage (Reddy et al., 2000). Moreover, the application of a chitosan-based layer on strawberries was proven to stimulate the production of defense proteins (Ban et al., 2018). The combination of chitosan and aloe vera fractions as edible coating materials has great potential in expanding the shelf life of blueberries (Vieiraa et al., 2016). Chitosan treatment has been reported to prolong the shelf life and control decay of cucumber, carrot, apple, citrus, kiwifruit, peach, pear, strawberry and sweet cherry (Ben et al., 2003). Spraying chitosan on vegetables has been shown to significantly reduce the severity of leaf spot diseases and increase the length of inflorescences in Dendrobium missteen (Deepmala et al., 2015). Chitosan at concentrations of 0.5% and 1% was reported to significantly decrease gray mold and blue mold caused by *B. cinera* and *P. expansum*, respectively, in tomato fruit stored at 25°C and 2°C (Liu et al., 2007). Molloy et al. (2004) investigated the in vitro effects of chitosan at concentrations of 1%, 2% and 4% (w/v) on Sclerotinia sclerotiorum. They also examined the effect of chitosan coating on sclerotinia rot of carrots (Daucus carota L.) held at 22°C. The combination of a hot water dip plus 2% chitosan resulted in significantly better disease reduction compared to papavas treated solely with synthetic fungicide for managing Anthracnose caused by Colletotrichum fructicola (Valencia et al., 2021).

Overall, chitosan-based interventions offer a sustainable and effective approach to post-harvest disease control, enhancing the quality and extending the shelf life of fruits and vegetables while reducing reliance on synthetic chemicals.

### Conclusion

Chitosan emerges as a multifaceted tool in sustainable agriculture, offering solutions across various domains critical for crop productivity and resilience. Its role extends beyond traditional disease management to encompass seed germination enhancement, abiotic stress tolerance, and promotion of crop growth and yield-contributing characters. Studies have demonstrated chitosan's efficacy in improving seed germination rates and uniformity, laying a strong foundation for robust crop establishment and early vigor. Moreover, its ability to mitigate the adverse effects of abiotic stressors such as drought and heat stress underscores its potential in safeguarding crop productivity under changing environmental conditions. In addition to its direct effects on plant physiology, chitosan's interactions with soil microbiota contribute to enhanced nutrient availability, root development and overall plant health, thereby facilitating optimal crop growth and yield formation. On the front of disease control, chitosan's antimicrobial properties offer a sustainable alternative to synthetic pesticides, effectively suppressing a wide range of fungal, bacterial, and viral pathogens. Its mode of action, including membrane disruption and induction of plant defense responses, provides a dual mechanism for combating diseases while priming plants for enhanced resistance against future infections.

### **Future perspectives**

Looking ahead, the future of chitosan in agriculture is characterized by exciting opportunities for innovation and growth:

- **Precision Agriculture:** Integrating chitosan into precision agriculture frameworks for optimized resource use and enhanced crop productivity.
- **Biotechnological Approaches:** Exploring genetic engineering and synthetic biology to enhance chitosan's efficacy and versatility.
- **Multi-Functional Formulations:** Developing multifunctional formulations combining chitosan with other bioactive compounds for synergistic effects.
- Environmental Applications: Leveraging chitosan for environmental remediation and sustainable land management practices.
- **Regulatory Frameworks:** Establishing clear regulatory standards to facilitate market access and ensure consumer confidence.
- **Knowledge Exchange and Capacity Building**: Promoting knowledge exchange and capacity building initiatives to empower stakeholders and broaden chitosan's reach.

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